X-100--.

On page 6, line 18, please delete "Triton X-100™" and insert therefor --TRITON X-100--.

On page 6, line 19, please delete each occurrence of "Triton X-100™" and insert therefor --TRITON X-100--.

On page 6, lines 19-20, please delete "Triton X-114™" and insert therefor --TRITON X-114--.

On page 6, line 20, please delete "Triton X-405™; Triton N-101™; Triton X-405™" and insert therefor --TRITON X-405; TRITON N-101; TRITON X-405--.

On page 7, line 7, please delete "Triton CG-110™, Triton XL-80N™" and insert therefor --TRITON CG-110, TRITON XL-80N--.

On page 7, line 8, please delete "Triton WR-1339™" and insert therefor --TRITON WR-1339 --.

In the Claims:

In claim 3, line 14, please delete "Tween 20™" and insert therefor --TWEEN 20--.

Remarks

I. Status of the Claims

The active claims in the application are 2-11 and 13-22.

II. Specification

The Examiner notes the use of trademarks TRITON X-100 and TWEEN 20, as well as others and requests that they be capitalized. Applicants have addressed this concern by capitalizing the various trademarks. Concerning the generic descriptions of IGEPAL, CA-360, TRITON X-100 and TWEEN 20, the Examiner's attention is respectfully drawn to page 6, lines 14-15. It is not necessary and certainly cumbersome to repeat this generic description in each place the trademark appears. The additional "TRITON" designations such as, for example, TRITON X-114 are designations known in the art and one of skill in the art would have no

difficulty understanding their meaning and no generic designation is necessary. Therefore, this objection should be withdrawn.

III. Rejection of Claims 2-11 and 13-22 Under 35 U.S.C. § 103

At page 2 of the Office Action mailed May 9, 2000, the Examiner rejected claims 2-11 and 13-22 under 35 U.S.C. § 103 as being unpatentable over Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1989)) in view of Chomczynski (U.S. Patent No. 5,346,994), newly cited Perlman (U.S. Patent No. 5,098,603) and also apparently DeBonville et al. (4,833,239). Applicant respectfully traverses the rejection.

Specifically, the Examiner states:

> Applicant acknowledges on page 2, third full paragraph, that Sambrook *et al.* discloses (sections 7.6-7.9) a method for isolating RNA comprising a non-ionic detergent, phenol and RNAse inhibitors and no chaotropic agent. Sambrook et al. does not teach use of solubilizers nor the use of EDTA to inhibit RNA degradation. However, Chomczynski does teach the use of phenol (30%-50%) with a solubilizer (3%-15%) at claim 8. Chomczynski does not teach the use of a phenol stabilizer. However, DeBonville et al. (4,833,239) does teach the use of both a stabilizer (8-hydroxyquinoline) and a solubilizer (isopropanol) at column 4, Example 1.
>
> Neither Chomczynski nor DeBonville teach the use of a chelating agent in extracting nucleic acids, however, Perlman teaches (col. 4, lines 37-51) the use of chelators such as EDTA when performing nucleic acid extractions with phenolic solutions to remove traces of divalent metal ions, some of which are known to catalyze the oxidation of phenol; moreover, Perlman further teaches that when even minute traces of metal ions are present, phenol solution is prone to catalytic oxidation, which adequately bridges the nexus between the differences in the prior art and the invention as claimed (and counters applicants's assertion in the instant specification on p.4, line 26 that the use of chelators to protect nucleic acids is novel).
>
> * * * *
>
> A person of ordinary skill in the art would have been motivated to combine the teachings of Perlman regarding the inclusion of a chelator with phenolic solutions with the nucleic acid extraction reagents of DeBonville and Chomczynski to prevent the oxidation of phenol by divalent metal ions.

Applicant disagrees.

To clarify the record, Applicant wishes to note that contrary to the Examiner's assertion, the specification at page 2 does not acknowledge that Sambrook discloses a method for isolating RNA that uses phenol. Applicant does note, however, that after using an RNA extraction buffer, Sambrook at page 7.7 does remove the proteins by further extracting once with an equal volume of phenol:chloroform.

The Examiner acknowledged that neither Chomczyniski nor DeBonville teach the use of a chelator. The Examiner then argues that because Perlman allegedly teaches the susceptibility of phenol to oxidation in the presence of traces of metal ions this permits combination of Perlman with the additionally cited art resulting in an obvious rejection. Applicant disagrees.

The rejection is incorrectly drawn to claim 21 and any claim dependent on claim 21. Claim 21 does not recite the use of a chelator and therefore should not be rejected in view of the art newly cited by the Examiner to support the use of a chelator. The claimed RNA isolation reagent of independent claim 21 contains at least one non-ionic detergent, at least one phenol and at least one phenol solubilizer, while the claimed RNA isolation reagent of independent claim 22 contains at least one non-ionic detergent, at least one phenol and at least one chelator.

The art cited by the Examiner, neither alone nor in combination, teaches or suggests an RNA isolation reagent containing the claimed combination of ingredients. Further, the cited art does not contain the requisite motivation to combine and fails to provide a reasonable expectation of success of obtaining the claimed invention. Both motivation and expectation of success are necessary to establish a *prima facie* case of obviousness. *In re Vaeck*, 20 U.S.P.Q. 1438, 1442 (Fed. Cir. 1991). The Examiner attempts to justify the use of Perlman in the combination of art because it "adequately bridges the nexus between the differences in the prior art and the invention as claimed." This is not a sufficient legal basis for combining of references. Thus, the rejection is based upon an improper legal premise and must be withdrawn.

At best, the Examiner appears to be arguing that there is a motivation to obtain the claimed invention. This is distinct from a motivation to combine references (*Uniroyal Inc. v. Rudkin-Wiley Corp.*, 837 F.2d 1044 (Fed. Cir. 1988). *Uniroyal* further stated that:

> [T]here must be some reason for the combination [of prior art references] other than the hindsight gleaned from the invention itself. Something in the prior art as a whole must suggest the desirability, and thus the obviousness, of making the

> combination. It is impermissible to use the claims as a frame and the prior art references as a mosaic to piece together a facsimile of the claimed invention.

Applicant respectfully submits that the Examiner has used hindsight to obtain the claimed invention by picking and choosing components of the invention from the art and then combining these components.

The Examiner's primary reference, Sambrook *et al.*, teaches a method of isolating RNA using an extraction buffer containing, *inter alia*, a detergent and RNAse inhibitor or vanadyl-ribonucleoside complex. The extraction buffer does not contain a phenol, phenol solubilizer, chelator or non-ionic detergent. It is noted that Sambrook *et al.* extract proteins with a phenol:chloroform reagent, however, that reagent is not used to extract RNA and is not a component of the RNA extraction buffer. *See* pages 7.6-7.7.

Chomczynski, the secondary reference, teaches an RNA, DNA and protein solvent solution containing chaotropic agents, a phenol, a phenol solubilizer and a buffer. The solution does not contain a non-ionic detergent or chelator.

Apparently, since Sambrook *et al.* and Chomczynski both disclose solutions employed in RNA isolation methods, the Examiner combined the two references in an attempt to recreate the claimed RNA isolation reagents. However, the Examiner failed to provide the motivation required to combine Sambrook *et al.* and Chomczynski and instead, reconstructed the invention by picking and choosing isolated teachings from the references. In doing so, the Examiner disregarded the fact that the references must be considered as a whole. One skilled in the art would not combine the Chomczynski solution for extracting RNA, DNA and proteins with the Sambrook *et al.* buffer for extracting RNA because, *inter alia*, the inclusion of a chaotropic agent, as taught by Chomczynski, in the Sambrook *et al.* buffer would cause co-isolation of polysaccharides so that the resulting RNA would not be in a purified form.

The third reference cited by the Examiner, DeBonville *et al.*, does not cure the deficiencies of Sambrook *et al.* or Chomczynski. DeBonville *et al.* is directed to methods of isolating DNA, *not* RNA. The DNA reagent composition contains, *inter alia*, phenol, isoamyl alcohol and 8-hydroxyquinoline. This composition does not contain the ingredients required to isolate RNA as set forth in the claimed reagents. Moreover, the reference is not analogous to the

claimed invention or to the Sambrook *et al.* and Chomczynski references. One skilled in the art would not use a DNA isolation reagent to isolate RNA. As noted by DeBonville *et al.*, a preferred DNA isolation method employs RNase A which degrades RNA (see col. 3, lines 62-64). Clearly, one isolating RNA would not follow the DNA isolation methods outlined in the DeBonville *et al.* patent and would not combine such methods with any RNA isolation method.

Perlman allegedly teaches a buffered and chelated phenol solution that is maintained under an inert gas atmosphere. This solution is reportedly useful to denature soluble proteins during the purification of nucleic acids. However, Perlman neither teaches nor suggests the use of a chelator as in the composition of claim 22. Specifically, at the section of the patent pointed out by the Examiner (Col. 4, lines 37-51) Perlman states that:

> In accordance with the invention, a divalent ion chelator such as ethylenediamine tetracetate (EDTA) is also been [sic] added to the buffered phenol at between about 0.1 and 10 mM but preferably at a concentration of approximately 1 mM.

Therefore, at best, the recitation pointed to by the Examiner teaches adding a *specific* concentration of EDTA, i.e. *0.1 to 10 mM* (preferably 1 mM) to a phenol solution, not to an RNA isolation reagent. Contrary to this, claim 22 recites "at least one chelator at a concentration of 0.02-0.25 M" (i.e. 20 mM-250 mM) in a composition with additional components. Nowhere is this concentration range of chelator suggested in Perlman. Further, even assuming *arguendo,* that the appropriate concentration range had been suggested there is no motivation whatsoever to combine the teachings of Perlman with the additionally cited art to arrive at the claimed invention.

The Examiner failed to point out any teaching in the art which would suggest which isolation reagent components are critical, or which would provide guidance leading to appropriate changes necessary to obtain the claimed invention or which combination of components should be used as an RNA isolation reagent. As a result, one skilled in the art would have *no* direction concerning how to successfully obtain the claimed invention. Contrary to the Examiner's viewpoint, simply because a piece of art, by itself, may allegedly teach *any* nucleic acid or protein isolation or a single component of an isolation reagent, this still fails to render the claimed invention obvious. Further, the motivation to combine references must be present

before combining the art, not after one has already decided what they wish the combination to show. Such motivation is lacking in the attempted combination of the art.

The art cited does not teach or suggest the claimed RNA isolation reagents either alone or in combination with each other. The primary reference, Sambrook *et al.*, fails to teach or suggest the claimed invention for the reasons indicated above. This failure is not remedied by the secondary references, Chomczynski, DeBonville *et al.* and Perlman. Thus, the combination of Sambrook *et al.* in view of Chomczynski, DeBonville *et al.* and Perlman does not render the claimed invention obvious.

Applicant submits that the rejection has been overcome. Withdrawal of the rejection is respectfully requested.

Conclusion

All of the stated grounds rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicant believes that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided. Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

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